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Dissociable Control of Impulsivity in Rats by Dopamine D2/3 Receptors in the Core and Shell Subregions of the Nucleus Accumbens

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Previous research has identified the nucleus accumbens (NAcb) as an important brain region underlying inter-individual variation in impulsive behavior. Such variation has been linked to decreased dopamine (DA) D2/3 receptor availability in the ventral striatum of rats exhibiting spontaneously high levels of impulsivity on a 5-choice serial reaction time (5-CSRT) test of sustained visual attention. This study investigated the involvement of DA D2/3 receptors in the NAcb core (NAcbC) and the NAcb shell (NAcbS) in impulsivity. We investigated the effects of a DA D2/3 receptor antagonist (nafadotride) and a DA D2/3 partial agonist (aripiprazole) infused directly into either the NAcbC or NAcbS of rats selected for high (HI) and low (LI) impulsivity on the 5-CSRT task. Nafadotride increased significantly the level of impulsivity when infused into the NAcbS, but decreased impulsivity when infused into the NAcbC of HI rats. By contrast, intra-NAcb microinfusions of aripiprazole did not affect impulsivity. Systemic administration of nafadotride had no effect on impulsive behavior but increased the number of omissions and correct response latencies, whereas systemic injections of aripiprazole decreased impulsive and perseverative behavior, and increased the number of omissions and correct response latencies. These findings indicate an opponent modulation of impulsive behavior by DA D2/3 receptors in the NAcbS and NAcbC. Such divergent roles may have relevance for the etiology and treatment of clinical disorders of behavioral control, including attention-deficit hyperactivity disorder and drug addiction.
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INTRODUCTION

Impulsivity is a multidimensional behavioral construct involving rash or risky behavior and a strong tendency toward spur-of-the-moment, poorly judged decisions and actions. Although it can be a beneficial personality trait, pathological manifestations of impulsivity are associated with a number of psychiatric disorders, including attention-deficit hyperactivity disorder (ADHD) (Winstanley *et al*, 2006) and substance use disorders (Brewer and Potenza, 2008, DSMIV American Psychiatric Association).

Recent conceptualizations have categorized impulsivity in terms of deficiencies in decision-making, inhibitory response control (for example, stopping) and in bridging delays to future rewards (Winstanley *et al*, 2006; Dalley *et al*, 2007; Pattij and Vanderschuren, 2008; Eagle *et al*, 2008; Robinson *et al*, 2009). Although the neural substrates of impulsivity are only partly understood, considerable evidence points to a significant involvement of the nucleus accumbens (NAcbs) (Dalley *et al*, 2008; Pattij and Vanderschuren, 2008), a forebrain region involved in the integration and expression of motivated behavior (Mogenson *et al*, 1980; Robbins and Everitt, 1996). It has recently been suggested that NAcb dopamine (DA) mediates high impulsivity in rats, potentially through abnormal modulation of NAcb function by DA D2/3 receptors. Thus, rats exhibiting a trait-like form of impulsivity—characterized by an inability to withhold a response to a cued visual stimulus on a 5-choice serial reaction time (5-CSRT) task (Bari *et al*, 2008)—show a reduced density of DA D2/3 receptors in the ventral striatum as measured by positron emission tomography (Dalley *et al*, 2007). However, the precise anatomical locus of this reduction in DA D2/3 receptors in the NAcb is unclear and could involve changes in one or more subregions of the ventral striatum, including especially the NAcb core (NAcbC) and NAcb shell (NAcbS) (Groenewegen *et al*, 1999; Zahm, 1999, 2000).

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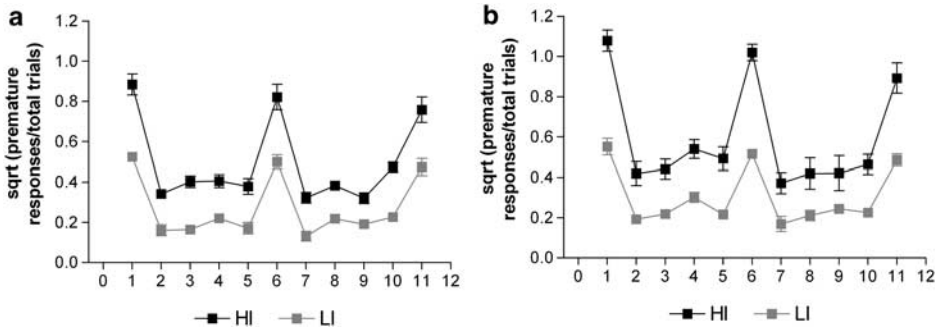

Figure 1 Levels of impulsivity expressed as square root of premature responses (premature responses/total trials) on the 5-CSRT task, during baseline sessions (2–5,7–10; ITI of 5 s) and long ITI sessions (1,6,11; ITI of 7 s), in HI and LI selected for intra-shell (group: $F(1,14) = 161.284$, $p \leqslant 0.0001$; session: $F(10,140) = 64.581$; $p \leqslant 0.0001$; group × session: $F(10,140) = 2.440$, $p \leqslant 0.001$) (a) or intra-core (group: $F(1,14) = 54.366$, $p \leqslant 0.0001$; session: $F(10,140) = 54.658$; $p \leqslant 0.0001$; group × session: $F(10,140) = 5.203$, $p \leqslant 0.0001$) (b) microinfusion experiments.

In this study, we therefore sought to investigate the role of DA D2/3 receptors in the NAcbC and NAcbS in mediating inter-individual differences in impulsive behavior on the 5-CSRT task. The effects of intra-NAcbC *vs* intra-NAcbS infusions of the DA D2/3 receptor antagonist, nafadotride, (Sautel *et al*, 1995) and the DA D2/3 partial agonist, aripiprazole, (DeLeon *et al*, 2004) were investigated and compared with systemic administration of these drugs in rats previously selected for high (HI) or low (LI) impulsivity. On the basis of previous findings showing that intra-NAcbC infusions of DA D2/3 receptor antagonists generally reduce impulsive behavior on the 5-CSRT task (Pattij *et al*, 2007; Pezze *et al*, 2009), we predicted that, in HI animals, nafadotride would similarly reduce impulsive behavior following its administration into the NAcbC. As HI and LI rats are likely to display differences in DA activity (Pattij and Vanderschuren, 2008), we expected aripiprazole to exert differential effects between HI and LI animals, as it can display both agonist and antagonist actions depending on DA levels (Deleon *et al*, 2004). Furthermore, although the role of DA D2/3 receptors in the NAcbS has not previously been investigated in the context of 'trait-like' impulsivity, we hypothesized on the basis of previous research that DA D2/3 receptors in the NAcbC and NAcbS may make dissociable contributions to the regulatory control of impulsive behavior (Pattij *et al*, 2007; Murphy *et al*, 2008).

MATERIALS AND METHODS

Subjects

A total of 96 male Lister Hooded rats (Charles River, Kent, UK) were used, housed under humidity and temperature-controlled conditions and an alternating light/dark cycle (red lights on from 0730 to 1930 hours). Rats weighing ~300 g at the start of the experiments were maintained at 85% of their free-feeding weight. Water was provided *ad libitum*. All experimental procedures were conducted in accordance to the UK Animals (Scientific procedures) Act 1986 (Home Office license number PPL 80/2234).

5-CSRT Training

Two groups of rats were trained in operant 5-CSRT task chambers (25 × 25 × 25 cm) controlled by WhiskerServer software (version 2.8) and FiveChoice client (version 2.6) (Cardinal and Aitken, 2001). Each daily session consisted of 100 discrete trials with stable performance being achieved after about 40 sessions. Animals were trained to enter a food magazine to initiate a trial. After an inter-trial-interval (ITI) of 5 s had elapsed, a brief light stimulus (0.5-s in duration) was pseudo-randomly presented in one of the five apertures. Following a nose poke in this aperture, (a 'correct' response) animals were rewarded with the delivery of one food pellet (45 mg Noyes dustless pellets) in the magazine. A nose-poke response in any of the adjacent apertures (an 'incorrect response'), as well as a failure to respond within 5 s after the onset of the stimulus ('omission'), resulted in no food delivery and a time-out period with the house light extinguished for 5 s. Nose pokes made during the ITI, that is, before the onset of the stimulus (or 'premature responses') were recorded as a measure of impulsivity, and resulted in a 5-s time-out and no food reward.

Screening for HI and LI

Following acquisition of the 5-CSRT task rats were challenged with three long ITI sessions to encourage impulsive responding, as described previously (Dalley *et al*, 2007; Belin *et al*, 2008; Economidou *et al*, 2009). Such sessions were presented at weekly intervals and consisted of a fixed long ITI of 7s. Subjects were ranked according to their level of impulsivity throughout the 3-week screening procedure and the highest and lowest 8 subjects selected as HI and LI rats, respectively. One group of HI and LI rats was selected for the intra-NAcbS infusions, which was subsequently also used for the systemic administration experiments (Figure 1a); another group of HI and LI animals was selected for the intra-NAcbC infusion experiment (Figure 1b).

Surgery

The HI and LI rats were anesthetized with ketamine (Ketaset, 100 mg/kg, intraperitoneally (i.p.); Vet Drug, Bury St Edmunds, UK) and xylazine (Rompun, 10 mg/kg, i.p., Vet Drug), and secured in a stereotaxic frame with the incisor bar set at −3.3 mm relative to the interaural line in flat skull position. Bilateral 22-gauge double-guide cannulae (Plastics

One, Sevenoaks, UK) were bilaterally implanted above either the NAcbC or the NAcbS, according to the following stereotaxic anterior–posterior (AP), mediolateral (ML), and dorsoventral (DV) coordinates: NAcbC: AP +1.5 mm, ML ±1.9 mm, DV −2.2 mm; NAcbS: AP +1.7 mm, ML ±0.75 mm, DV −2 mm. AP and ML coordinates were taken from bregma, DV coordinates from skull surface (Paxinos and Watson, 1998). Cannulae were secured to the skull with dental acrylic and stainless steel screws and occluded by a stylet. After surgery, animals were allowed to recover for a week.

Microinfusions

Following reestablishment of stable performance on the 5-CSRT task, intracerebral microinfusions of nafadotride and aripiprazole were carried out in HI and LI rats 5 min before behavioral testing. One rat from each group of HI animals was removed from the study because of unstable performance after surgery. The infusion experiments were run over a 3-day cycle, starting with an initial baseline session. On day 2, animals received an infusion of drug or vehicle ('veh') before testing. On day 3, animals were not tested and remained in their home cages. The microinfusions were delivered through a 28-gauge bilateral injector (Plastics One, Roanoke, USA) inserted through the guide cannula and extending 4.5 mm (NAcbC) or 5 mm (NAcbS) beyond the tip of the guide. The injector was left in place for 1 min before each 1 min infusion (0.5 μl) and a further 1 min afterwards to allow sufficient time for the drug to diffuse into the surrounding tissue. Rats received two previous habituation sessions in the same testing conditions, separated by 2 days of baseline training. During the first habituation session, the injector was inserted through the guide cannula and left in place for 1 min. During the second habituation session, rats received an infusion of vehicle. The first drug infusion was given after a further 2 days of baseline training. Rats first received infusions of nafadotride (veh, 0.1 and 0.3 μg) and aripiprazole (veh, 0.03, 0.1, and 0.3 μg) according to a randomized Latin-square design with a wash-out period of 1 week. The doses were selected according to previous research (Barik and de Beaurepaire, 2005) and pilot experiments. All drug testing was performed with sessions comprising a fixed ITI of 5 s.

Systemic Drug Administration

Nafadotride and aripiprazole were administered by systemic intraperitoneal injection in HI and LI rats that had previously received intra-NAcbS microinfusions. Systemic injections were given 1 week after the last intracerebral infusion on a 3-day cycle as described above. Nafadotride and aripiprazole were injected 20 min before behavioral testing according to a Latin-square design (veh, 1 and 3 mg/kg) with a wash-out period of 1 week between both drugs. The doses were based on previously published research (Boulougouris *et al*, 2008; Nordquist *et al*, 2008; St Onge and Floresco, 2009). All drug testing was performed with sessions comprising a fixed ITI of 5 s.

Drugs

Aripiprazole (Toronto Research Chemicals, North York, Canada) was dissolved in 2% glacial acetic acid and 30% dimethyl formamide in distilled deionized water. The pH was adjusted to 5.5 with 0.1 M NaOH. Nafadotride (Tocris Cookson, Bristol, UK) was dissolved in 1 M HCl and normal saline and the pH adjusted to 6 with 0.1 M NaOH. Both drugs were injected in a volume of 1 ml/kg. All drugs were aliquoted after preparation and frozen at −80°C.

Histological Assessment of Cannulae Placements

Animals received an overdose of sodium pentobarbital (1.5 ml per rat, i.p., Dolethal 200 mg/ml, Rhone-Merieux, Athens, USA), and perfused transcardially with 0.01 M PBS followed by 4% paraformaldehyde. The brains were removed and post-fixed in 4% paraformaldehyde overnight. The brains were transferred into a 20% sucrose solution in 0.01 M PBS and left overnight before being sectioned into 60-μm coronal sections with a freezing microtome. Every third section was mounted and stained with Cresyl Violet. Cannulae placements were verified under a light microscope and mapped onto standardized coronal sections of the rat brain (Paxinos and Watson, 1998).

Statistical Analysis

Two-way repeated-measures ANOVAs were used to analyze the effects of intra-NAcb microinfusions on 5-CSRT task performance in HI and LI rats (NAcb subregion × drug dose). For the analysis of the effects of intra-NAcb nafadotride, three-way repeated-measures ANOVAs were additionally performed (group × NAcb subregion × drug dose). The effects of systemic drugs administration were assessed by two-way repeated-measures ANOVA (group-drug). If the sphericity assumption was violated, the Greenhouse–Geisser epsilon test was applied to calculate a more conservative p-value for each F ratio. On confirmation of significant main effects, differences among individual means were analyzed using the Duncan's *post-hoc* test. Significant violations of homogeneity of variances across the HI and the LI groups and of normality were corrected using square root transformations. For all analyses, the significance level was $\alpha = 0.05$.

RESULTS

Histology

Figure 2 shows the positions of the injector tips in the NAcbC and NAcbS. In total, eight rats were excluded from the study (two HI and six LI) because injector cannulae were positioned outside the target areas. There was no gross tissue damage in the local vicinity of the injector tracks.

Intra-NAcb Nafadotride Infusions

The effects of intra-NAcb infusions of nafadotride on impulsive responding and other behavioral measures on the 5-CSRT task are shown in Figure 3 and Supplementary Table 1 (see Supplementary online material). In HI animals,

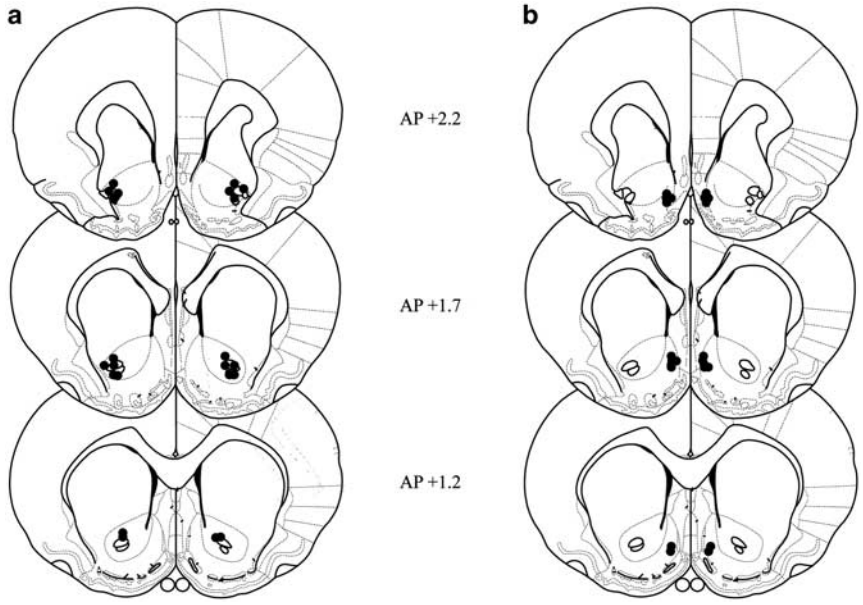

Figure 2 Schematic representations of injector tips in the shell ($n = 11$) (a) and the core ($n = 12$) (b) of the NAcb. Reconstructed from Paxinos and Watson (1998).

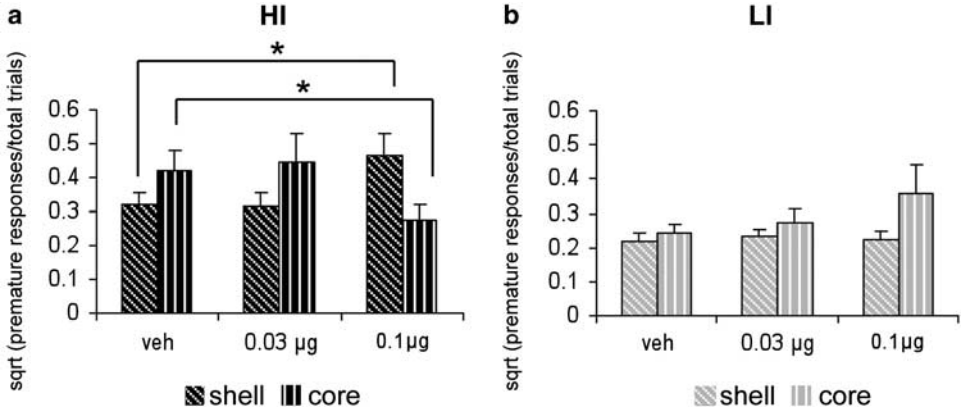

Figure 3 Effects of intra-NAcb shell (diagonal stripes) or core (vertical stripes) microinfusions of nafadotride (0, 0.03, or 0.1 µg per 0.5 µl) on impulsivity in HI (a) and LI (b) rats on the 5-CSRT task. Impulsivity is expressed as square root of premature responses (premature responses/total trials). For the shell experiment, $n = 6$ HI and $n = 5$ LI rats. For the core experiment, $n = 6$ HI and $n = 5$ LI. Doses are expressed in µg/0.5 µl. Each bar represents the mean ± SEM. *$p \leqslant 0.05$.

nafadotride had opposite effects on the level of premature responses depending on the Nacb subregion into which it was infused (group × NAcb subregion interaction: $F(2,20) = 7.4125$, $p \leqslant 0.01$, Figure 3a). *Post-hoc* analysis showed that impulsivity was significantly increased in HI rats when nafadotride was infused into the NAcbS at the highest dose tested (0.1 µg *vs* veh: $p \leqslant 0.05$, $n = 6$). By contrast, microinfusions of nafadotride into the NAcbC significantly decreased impulsivity in HI animals at the highest dose tested (0.1 µg *vs* veh: $p \leqslant 0.05$, $n = 6$). In LI animals, microinfusions of nafadotride into either the NAcbS ($n = 5$) or the NAcbC ($n = 5$) had no significant effect on premature responding (Figure 3b).

The double dissociation of the effects of microinfusions of nafadotride on impulsivity depending on both the Nacb subregion and the group of animals (HI or LI) tested was further confirmed by three-way ANOVA (group × drug × Nacb subregion interaction: $F(2,36) = 7.227$, $p \leqslant 0.01$; group: $F(1,18) = 9.790$, $p \leqslant 0.01$). There were no significant effects of intra-NAcbS and intra-NacbC nafadotride on choice accuracy, omission, perseveration, correct response latencies and magazine latencies (see Supplementary Table 1).

Intra-NAcb Aripiprazole Infusions

The effects of intra-NAcb infusions of aripiprazole on impulsive responding and other behavioral measures on the 5-CSRT task are shown in Figure 4 and Supplementary Table 2 (see Supplementary online material). Microinfusions of aripiprazole into either the NAcbS or the NAcbC

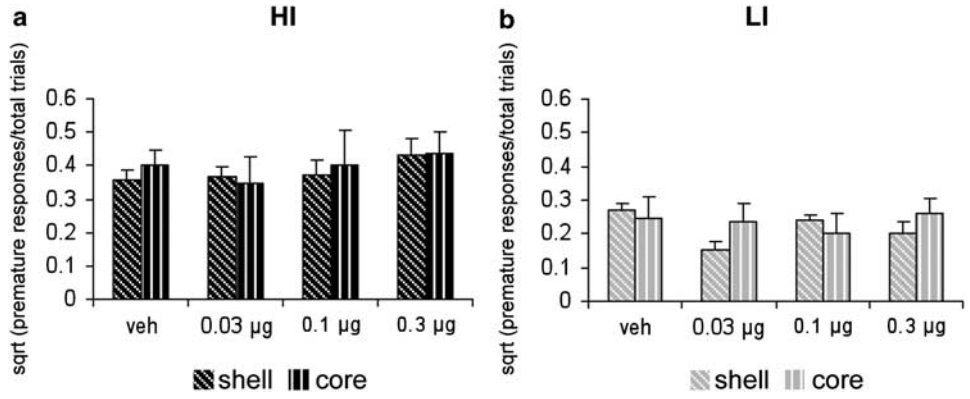

Figure 4 Effects of intra-NAcb shell (diagonal stripes) or core (vertical stripes) microinfusions of aripiprazole (0, 0.03, 0.1, or 0.3 µg per 0.5 µl) on impulsivity in HI (a) and LI (b) rats on the 5-CSRT task. Impulsivity is expressed as square root of premature responses (premature responses/total trials). For the shell experiment, $n = 6$ HI and $n = 5$ LI rats. For the core experiment, $n = 6$ HI and $n = 6$ LI. Doses are expressed in µg/0.5 µl. Each bar represents the mean ± SEM.

did not significantly affect impulsive responding in either HI (NAcbS: $n = 6$; NAcbC: $n = 6$) (Figure 4a) or LI (NAcbS: $n = 5$; NAcbC: $n = 6$) rats (Figure 4b).

No significant effects of intra-NAcbS and intra-NacbC aripiprazole microinfusions were observed on choice accuracy, omission, perseveration, correct response latencies, and magazine latencies in either HI or LI animals (see Supplementary Table 2).

Systemic Nafadotride Administration

The effects of systemic nafadotride on 5-CSRT task performance are shown in Figure 5. Nafadotride significantly increased the number of omissions (drug: $F(2,26) = 10.123$, $p \leqslant 0.001$, Figure 5d), in both HI ($n = 7$) and LI animals ($n = 8$) (group × drug: $F(2,26) = 0.830$, NS). *Post-hoc* analysis showed that this effect was attributable to the highest dose tested (3 mg/kg *vs* veh: $p \leqslant 0.0001$). Nafadotride also significantly increased correct response latencies ($F(2,26) = 12.101$, $p \leqslant 0.001$, Figure 5e) in both HI and LI rats (group × drug: $F(2,26) = 0.198$, NS). Correct response latencies were significantly increased by nafadotride injections at the highest dose tested (3 mg/kg *vs* veh: $p \leqslant 0.0001$). Systemic administration of nafadotride had no effect on impulsive responding, perseveration, attentional accuracy, and magazine latencies (Figures 5a–c and f).

Systemic Aripiprazole Administration

The effects of systemic aripiprazole on 5-CSRT task performance are shown in Figure 6.

Aripiprazole significantly decreased impulsivity in both HI ($n = 7$) and LI rats ($n = 8$) (drug: $F(2,26) = 8.200$, $p \leqslant 0.01$; group: $F(1,13) = 9.989$, $p \leqslant 0.01$; group × drug: $F(2,26) = 2.495$, NS; Figure 6a). *Post-hoc* analyses showed that this effect was significant at both doses tested (1 mg/kg *vs* veh: $p \leqslant 0.05$; 3 mg/kg *vs* veh: $p \leqslant 0.01$). Systemic aripiprazole also significantly reduced perseverative responding (drug: $F(2,26) = 4.494$, $p \leqslant 0.05$; group × drug: $F(2,26) = 2.987$, NS; Figure 6b). *Post-hoc* analyses revealed that perseverative responding was significantly decreased at both doses tested (1 mg/kg *vs* veh: $p \leqslant 0.05$; 3 mg/kg *vs* veh: $p \leqslant 0.05$). In addition, aripiprazole significantly increased omissions (drug: $F(2,26) = 21.373$, $p \leqslant 0.0001$; group × drug: $F(2,26) = 0.471$, NS; Figure 6d), at both doses tested (1 mg/kg *vs* veh: $p \leqslant 0.01$; 3 mg/kg *vs* veh: $p \leqslant 0.001$). Aripiprazole also increased correct response latencies (drug: $F(2,20) = 4.170$, $p \leqslant 0.05$; group × drug: $F(2,20) = 1.250$, NS; Figure 6e), an effect attributable to the highest dose ($p \leqslant 0.05$). Systemic administration of aripiprazole had no significant effect on attentional choice accuracy (Figure 6c) and magazine latencies (Figure 6f).

DISCUSSION

This results provide new insights into the neurobiological basis of impulsivity. Nafadotride, a DA D2/3 receptor antagonist, exerted an impulsivity state-dependent, dissociable effect in the NAcbS and NAcbC, increasing the level of impulsivity when infused into the NAcbS, but decreasing it when infused into the NAcbC, selectively in HI animals. Systemically administered nafadotride had no effect on impulsive behavior but increased omission levels and correct response latencies. By contrast, aripiprazole, a DA D2/3 receptor partial agonist, had no effect on impulsive behavior when infused into either the NAcbS or the NAcbC, but decreased impulsive and perseverative behavior, whereas increasing errors of omission and lengthening correct response latencies, when administered systemically.

The NAcb has been widely associated with impulsive behavior in humans and rodents (Cardinal *et al*, 2001; Aron *et al*, 2007; Pattij and Vanderschuren, 2008). We have reported that spontaneously high impulsive rats on the 5-CSRT task exhibit a decrease in DA D2/3 receptor availability in the ventral striatum, including the NAcb (Dalley *et al*, 2007). Dopaminergic function has been broadly implicated in disorders of impulse control mainly on the basis of the evidence that psychostimulants are effective in ADHD (Solanto, 2002; Winstanley *et al*, 2006). In this study, systemic administration of DA D2/3 agents either had no effect, or nonselectively affected impulsivity, whereas increasing omissions and lengthening correct latencies, suggesting a rather general effect on locomotor

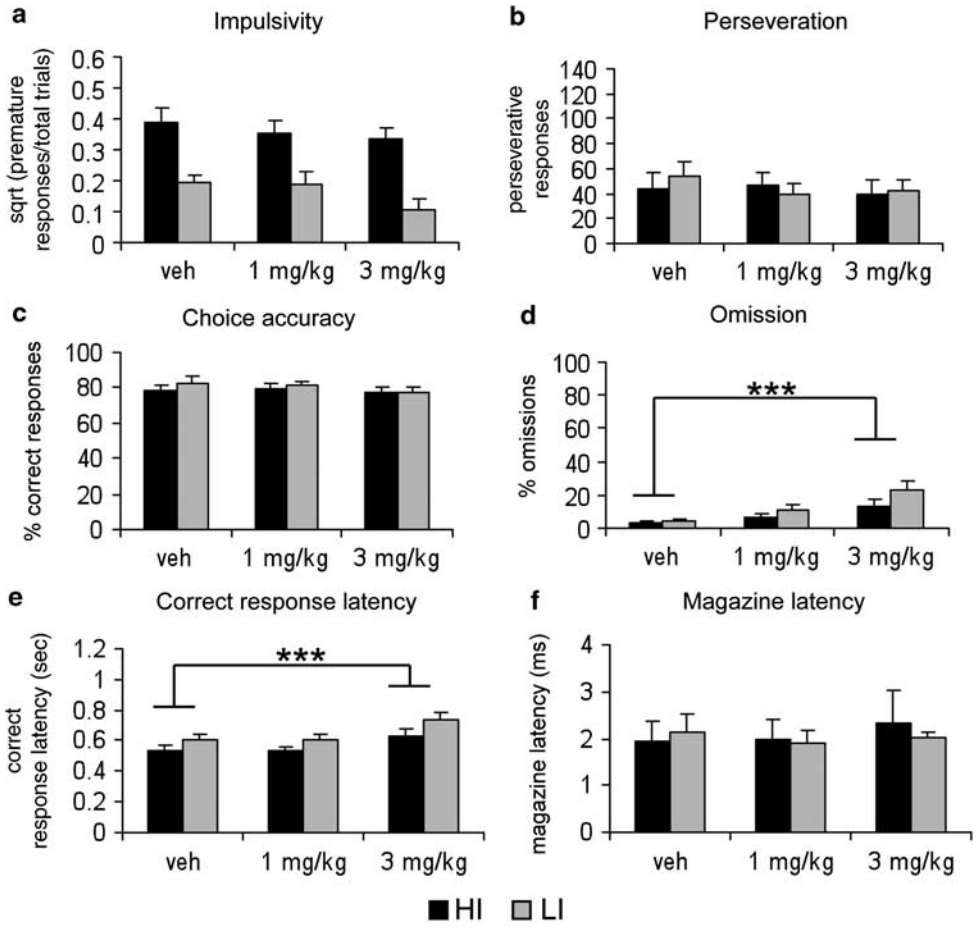

Figure 5 Effects of systemic nafadotride administration on 5-CSRT task performance in HI (black bars, $n = 7$) and LI rats (gray bars, $n = 8$). Doses are expressed in mg/kg. Each bar represents the mean ± SEM. (a) Impulsivity expressed as square root of premature responses (premature responses/total trials). (b) Perseveration expressed as a number of perseverative nose pokes. (c) Choice accuracy expressed as a percentage of correct responses. (d) Omission expressed as a percentage of omissions. (e) Correct response latency expressed in sec. (f) Magazine latency expressed in seconds. ***$p \leqslant 0.001$.

or motivational functions. However, we provide functional evidence that trait-like impulsivity is nonetheless dependent on intra-NAcb DA neurotransmission involving DA D2/3 receptors. As an increasing or decreasing DA neurotransmission enhances or reduces, respectively, impulsivity on the 5-CSRT task (Cole and Robbins, 1989; Pattij and Vanderschuren, 2008), it could be hypothesized that HI rats show increased DA activity as compared with LI animals, as a consequence of too few DA D2/3 receptors in the NAcb (Dalley *et al*, 2007) functioning as autoreceptors (Viggiano *et al*, 2003). Indeed, impulsivity on the 5-CSRT task has been associated *in vitro* with high and low DA release in the NAcbS and NAcbC, respectively (Diergaarde *et al*, 2008). Accordingly, these results support a subregion-dependent dopaminergic basis for inter-individual differences in impulsivity, which may depend on opponent dopaminergic modulation of the NAcb core and shell subregions.

In HI animals, nafadotride decreased impulsivity when infused into the NAcbC. This result is consistent with recent studies showing that intra-NAcbC infusions of D2-like receptor antagonists decrease impulsivity when high levels of this behavior are induced, either by PFC lesions (Pezze *et al*, 2009) or by amphetamine (Pattij *et al*, 2007). NAcb D2-like DA receptors have been suggested to regulate behavioral control via cortical and limbic influences possibly through a gating mechanism (Floresco, 2007). Within the NAcb, DA can either enhance or decrease glutamate-induced activity of medium spiny neurons, by actions at D2-like receptors (Yang and Mogenson, 1986; Goto and Grace, 2005). Thus, decreased stimulation of NAcb D2/3 receptors by tonic DA release has been shown to facilitate PFC inputs, and unilateral inactivation of the PFC combined with NAcb D2/3 receptor stimulation decreases inhibitory control during goal-directed behavior (Goto and Grace, 2005). Hence, in this study, blockade of NAcbC DA D2/3 receptors in HI rats may decrease impulsive responding through a facilitation of PFC glutamatergic inputs, thereby increasing PFC control over behavior. This result suggests that, in HI individuals, intra-NAcbC D2/3-mediated DA neurotransmission that negatively modulates PFC glutamatergic inputs would be in a constitutively hyperactive state.

By contrast, intra-NAcbS nafadotride exacerbated impulsive responding in HI animals, suggesting that the impulsive trait is associated with constitutively hypoactive

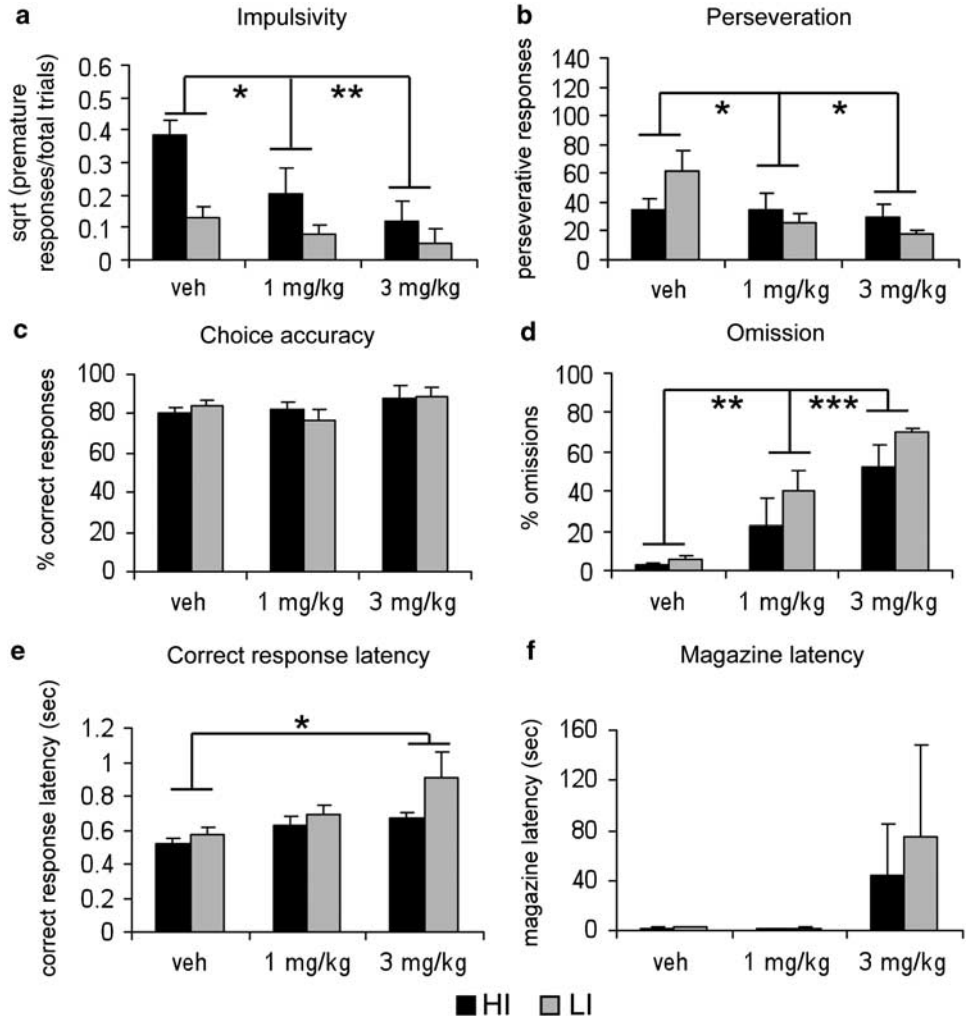

Figure 6 Effects of systemic aripiprazole administration on 5-CSRT task performance in HI (black bars, $n = 7$) and LI rats (gray bars, $n = 8$). Doses are expressed in mg/kg. Each bar represents the mean ± SEM. (a) Impulsivity expressed as square root of premature responses (premature responses/total trials). (b) Perseveration expressed as a number of perseverative nose pokes. (c) Choice accuracy expressed as a percentage of correct responses. (d) Omission expressed as a percentage of omissions. (e) Correct response latency expressed in seconds. (f) Magazine latency expressed in seconds. $^{*}p \leqslant 0.05$, $^{**}p \leqslant 0.01$, $^{***}p \leqslant 0.001$.

D2/3-mediated DA neurotransmission in this subregion. Thus, the proposed D2-like receptor-mediated gating mechanism underlying behavioral output might be less efficient in HI individuals because D2/3-mediated DA neurotransmission in the NAcbS and NAcbC is dysregulated. The apparent lack of effect of systemic nafadotride on impulsivity might result from these opposing actions in both the NAcbS and NAcbC, and not because an insufficient dose of nafadotride was used (see also Boulougouris *et al* (2008); St Onge and Floresco (2009)). It is also conceivable that brain regions other than the NAcb mediate in part the effects of the systemically administered drugs, potentially through opponent interactions between the PFC and striatum. For example, previous research has strongly implicated the anterior cingulate cortex (Muir *et al*, 1996), infralimbic cortex (Chudasama *et al*, 2003), and dorsomedial striatum (Rogers *et al*, 2001) in the regulation of impulsive behavior.

Previously, it has been shown that intra-NAcbC infusions of the DA D2/3 receptor antagonists sulpiride (Pezze *et al*, 2009) and eticlopride (Pattij *et al*, 2007) result in increased omissions and correct response latencies on the 5-CSRT task without affecting impulsive behavior. By contrast, in this study, intra-NAcb infusions of nafadotride generally only affected impulsive responding. As sulpiride and eticlopride are both more selective for DA D2 than DA D3 receptors (Vallone *et al*, 2000; Levant, 1997), and nafadotride is more selective for DA D3 than DA D2 receptors (Sautel *et al*, 1995), attentional performance and impulsivity may be differentially modulated by DA D2 and DA D3 receptors, respectively. Consistent with this notion, nafadotride increased omissions and response latencies at doses that would be expected to block DA D2 receptors (Levant and Vansell, 1997).

In this study, intra-NAcbS or NacbC infusions, as well as systemic injections of the DA D2/3 partial agonist aripiprazole, did not modify levels of impulsivity in HI and LI rats. Aripiprazole is a partial DA receptor agonist with high affinity for both DA D2 and D3 receptors (deLeon *et al*, 2004). On the basis of the hypothesis that HI and LI

rats may show hyper- and hypo-DA activity, respectively (Pattij and Vanderschuren, 2008), we had postulated that aripiprazole may have different effects on impulsive behavior in HI and LI rats. In fact, several studies have shown that aripiprazole can have either agonist or antagonist properties at DA D2/3 receptors depending on the level of DA activity (Deleon *et al*, 2004). The absence of an effect of aripiprazole on impulsivity in this study might suggest that inter-individual differences in impulsivity on the 5-CSRT task are not related to major differences in DA release, consistent with evidence that HI rats do not show higher striatal DA release than non-impulsive rats (Dalley *et al*, 2007). The lower number of DA D2/3 receptors in the ventral striatum of HI rats would hence not seem to be associated with an impairment in presynaptic function, which would directly affect DA tone (Benoit-Marand *et al*, 2001), but rather to a change in postsynaptic activity. However, previous research has shown that electrically evoked DA release is increased and decreased in the NAcbS and NAcbC, respectively, in HI compared with LI rats (Diergaarde *et al*, 2008). Therefore, the absence of an effect of aripiprazole on impulsive action might also be due to a concomitant action on pre- and postsynaptic DA D2/3 receptors (Deleon *et al*, 2004), in contrast to nafadotride, which seems preferentially to block postsynaptic receptors at low doses (Griffon *et al*, 1995).

These data revealing dissociable roles of DA D2/3 receptors in the NAcbS and NAcbC in impulsive behavior are compatible with previous evidence showing contrasting effects on impulsivity of NAcb shell *vs* core deep brain stimulation on a reaction-time task, with impulsivity being decreased by NAcbC, but increased by NAcbS stimulation (Sesia *et al*, 2008). In addition, NAcbC lesions were shown to potentiate, whereas NAcbS lesions were found to attenuate, amphetamine-induced increases in impulsive behavior on a forced choice task (Murphy *et al*, 2008). Thus, interactions between NAcbS- and NAcbC-dependent mechanisms likely mediate the expression of high impulsivity on the 5-CSRT task. The precise direction of this interaction is unknown but may be relevant to the hierarchical or cascading anatomical arrangement of the ventral midbrain DA system previously described in primates (Haber *et al*, 2000) and rats (Ikemoto, 2007). Thus, the NAcbS projects to the ventral tegmental area (VTA) that reciprocally projects to the NAcbS, but also to the NAcbC. The NAcbC influences VTA and substantia nigra neurons in turn, and thereby modulates more dorsal structures of the striatum to control behavioral output. Hence, impaired DA D2/3-mediated neurotransmission within the NAcbS might affect the entire DA cascading circuitry, eventually altering NAcbC activity and consequently behavioral output. Such an influence of the NAcbS over NAcbC functioning in the control of behavior has already been implicated in the context of latent inhibition (Weiner *et al*, 1999) and neural plasticity (Mameli *et al*, 2009).

Trait-like impulsivity on the 5-CSRT task has recently been identified as a vulnerability marker for cocaine (Dalley *et al*, 2007; Belin *et al*, 2008; Economidou *et al*, 2009) and nicotine (Diergaarde *et al*, 2008) addiction. A role of the NAcbS has been identified in the reinforcing and stimulant action of cocaine, whereas the NAcbC has been shown to be involved in the acquisition of cocaine-seeking behavior (Ito *et al*, 2004). Moreover, the circuitry connecting the NAcbC and the dorsal striatum has been shown to mediate the establishment of cocaine-seeking habits (Belin and Everitt, 2008). As striatal DA D2/3 receptors have also been linked to drug seeking (Pilla *et al*, 1999; Nader *et al*, 2006; Volkow *et al*, 2009), a putative dysregulation of DA neurotransmission involving DA D2/3 receptors in the NAcbS and the NAcbC of HI rats might therefore also account for their propensity to stimulant drug addiction.

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